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Pseudo-Outbreak of *Bordetella parapertussis* Caused by Contaminated Swabs in the Netherlands

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An increase in positive *Bordetella parapertussis* tests among patients in a teaching hospital in the Netherlands resulted in enhanced infection control and microbiological surveillance. Further analysis revealed that batches of contaminated nasopharyngeal swabs were associated with a pseudo-outbreak, resulting in incorrect diagnoses, antimicrobial treatments, isolation precautions, and public health notifications.

We report a pseudo-outbreak of *Bordetella parapertussis* in the Department of Pediatrics in Rijnstate, an 809-bed teaching hospital in the Netherlands. The department provides level II care to infants, neonates, and preterm infants. In March 2021, we diagnosed *B. parapertussis* in 3 infants hospitalized for respiratory symptoms by using an in-house PCR against insertion sequences (IS) IS481 and IS1001 (1). During calendar week 21 (Figure), we identified more *B. parapertussis* cases in the same department, bringing the total case count to 5 in neonates, 1 in a toddler, and 6 in infants. Several of these patients were born prematurely.

PCR-positive case-patients had pertussis-like complaints, and we confirmed *B. parapertussis* in the patients or their siblings. We traced all positive tests to the Department of Pediatrics. Because we suspected nosocomial transmission, we started contact tracing investigations among parents and healthcare workers (HCWs) and identified *B. parapertussis* in another 4 patients and in 3 HCWs.

Cases among HCWs were particularly unexpected. Because of the coronavirus disease (COVID-19) pandemic, all HCW were using type IIR surgical masks and keeping ≥1.5 m distance from each other. In addition, all patients had private rooms, and we observed no increase in other respiratory pathogens.

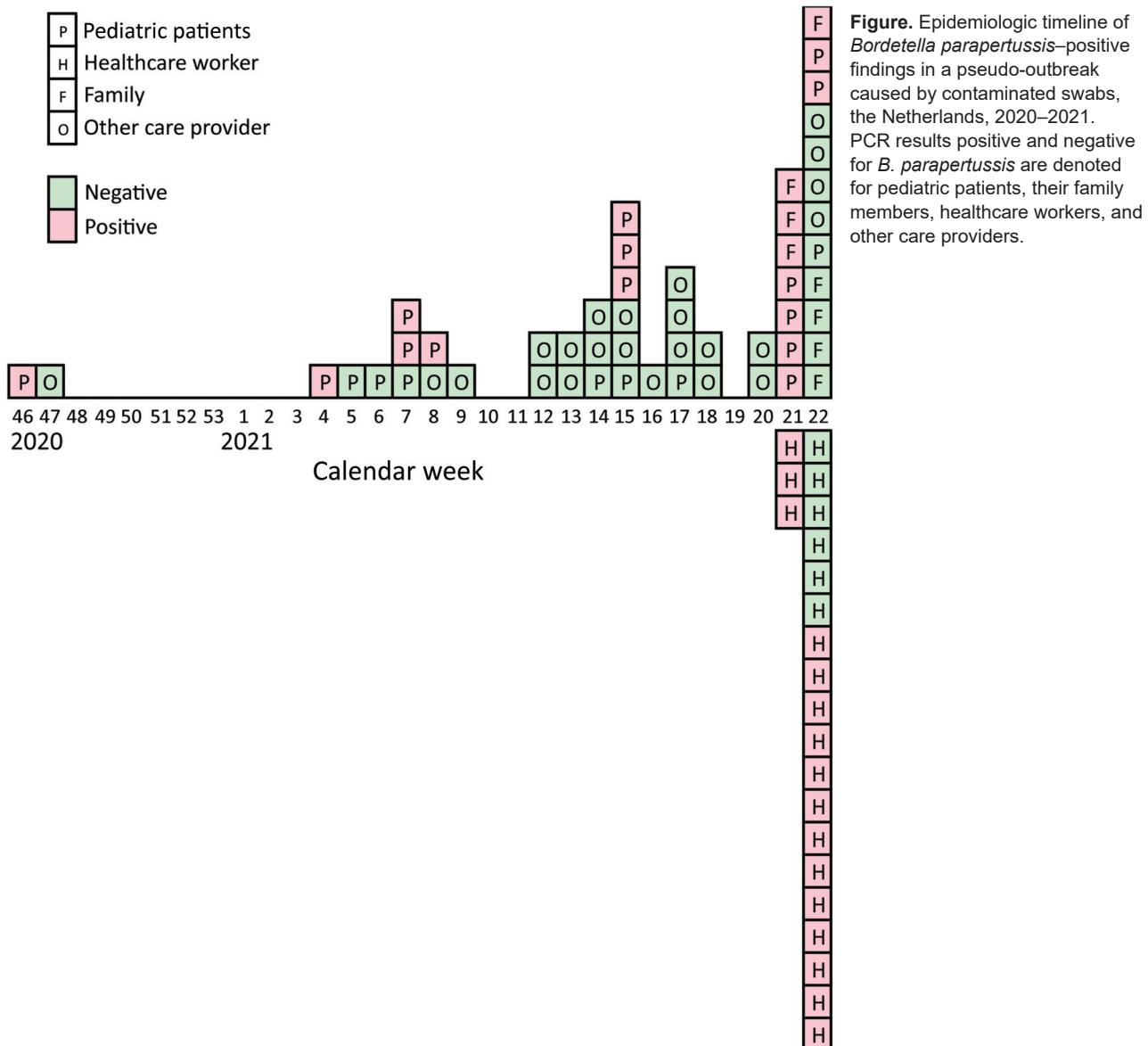


Figure. Epidemiologic timeline of *Bordetella parapertussis*-positive findings in a pseudo-outbreak caused by contaminated swabs, the Netherlands, 2020–2021. PCR results positive and negative for *B. parapertussis* are denoted for pediatric patients, their family members, healthcare workers, and other care providers.

Because we discovered additional *B. parapertussis*-positive cases, we upgraded HCW masks to FFP1, the recommended type for pertussis (2). We also confirmed instructions regarding continued HCW social distancing, including during lunch breaks, to prevent further *B. parapertussis* spread. We then implemented extended screening for asymptomatic cases among all HCWs and relatives of *B. parapertussis*-positive case-patients. Among 22 HCWs tested, 72% (16/22) tested *IS1001*-positive.

Parallel to actions in the clinic, we checked the possibility of laboratory contamination. The laboratory uses several controls to confirm sensitivity and specificity of assays; all controls consistently showed correct results. Swipe-tests did not reveal

contaminated surfaces or equipment. All cases had relatively high cycle threshold (C_t) values (median C_t 35), as seen with prior *B. parapertussis* results from the laboratory ($n = 17$). In addition, 2 other laboratories confirmed *B. parapertussis* in original clinical samples and DNA eluates by targeting diverse regions of the *IS1001* gene using an in-house PCR (1) or Real Accurate Quadruplex Bordetella PCR (PathoFinder, <https://www.pathofinder.com>). Confirmatory PCR tests also had high C_t values.

Finally, we tested unused ESwab 483CE nasopharyngeal swabs (Copan, <https://www.copanusa.com>), which included a flocked swab and 1 mL of liquid Amies medium in a plastic, screw cap tube. Liquid Amies media from 7 batches (1 sample per

batch) and flocked tips from 2 batches (2 tips per batch) were available for testing. All liquid Amies media were PCR-negative, but both batches of flocked swab tips were PCR-positive for *IS1001*. Moreover, 2 flocked tips were placed in 0.5 mL of Milli-Q water (Millipore, <https://www.emdmillipore.com>), a 4-fold higher concentration than for standard diagnostic tests. In the higher concentration, we saw lower C_t values ($C_t \approx 35$) compared with regular diagnostic tests ($C_t \geq 37$).

We retested all 23 PCR-positive HCWs by using individually packaged 503CS01 flocked swabs (Copan) from a PCR-negative batch; 22 HCWs tested PCR-negative and 1 tested PCR-positive. Upon re-examination, we found that testing for the positive HCW case was not performed with an individually packaged swab provided by the laboratory but an ES-wab from the suspect batch. Although unintentional, this case proved that the *B. parapertussis* could be traced to *IS1001*-positive nasopharyngeal swabs tips. No *B. parapertussis* could be cultured, which aligns with the notion that the swabs are gamma-irradiated after packaging. Gamma irradiation kills bacteria but does not affect DNA.

We alerted clinical and molecular microbiologists in the Netherlands, the supplier, and the Health Inspectorate regarding swabs contaminated with *B. parapertussis* *IS1001*-containing DNA. Subsequently, >6 laboratories in the Netherlands recognized and reported false-positive *B. parapertussis* to the National Institute of Public Health and the Environment. Contamination appeared to be associated with specific ESwab batch numbers (Appendix, <https://wwwnc.cdc.gov/EID/article/28/4/21-2097-App1.pdf>), which explains why the pseudo-outbreak focused on 1 department in our hospital. The contamination was confirmed by the manufacturer, but the source was not disclosed.

B. parapertussis can cause pertussis-like symptoms, although symptoms usually are milder and occur less frequently than with *B. pertussis* (3). Each year, ≈6,400 *B. pertussis* cases are notified in the Netherlands based on culture, PCR, or serology, but only 26 *B. parapertussis* cases are notified (Appendix Figure). During 2020–2021, the COVID-19 pandemic and associated social distancing measures caused a large decrease in reported *B. pertussis* cases. Of note, *B. parapertussis* reports did not diminish during this period (Appendix), possibly because testing strategies changed, contaminated swabs were already circulating, or both.

Our report illustrates the importance of critically evaluating microbiological results lacking clinical and epidemiologic clues. We were confronted with a growing number of neonatal patients and HCWs with unexplained *B. parapertussis*-positive tests. Because these tests are requested infrequently, it took months before contamination with *IS1001*-like DNA in nasopharyngeal swabs became clear. Clinicians and public health agencies should be aware of the possibility of false-positive microbiology results and consider contaminated products when unexplainable results are found.

Acknowledgments

We thank our colleagues in the laboratory, the Department of Pediatrics, Infection Prevention, and the Safety and Public Health Service Gelderland Midden for their contributions to this work. We thank Canisius Wilhelmina Hospital (Nijmegen, the Netherlands) and the University Medical Center Utrecht (Utrecht, the Netherlands) for assistance in confirming contamination on the swabs and the Working Group on Molecular Diagnostics of Infectious Diseases for the national survey on contaminated swabs. We also thank Dimphey van Meijeren for collecting the data on the notifications to the Center for Infectious Disease Control.

About the Author

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Pseudo-Outbreak of *Bordetella parapertussis* Caused by Contaminated Swabs, the Netherlands

Appendix

Notifications

Notifications were sent by the Ministry of Health, the Netherlands, to members of Labinf@ct regarding nasopharyngeal swabs contaminated with *Bordetella parapertussis* insertion sequence 1001 (*IS1001*) DNA during 2020–2021. Notifications were provided in Dutch and English.



Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

Labinf@ct: B. parapertussis (2)

20 juli 2021

In dit bericht:

- Meerdere swabs betrokken bij fout-positief resultaat in een *B. parapertussis*-PCR: 8 lotnummers e-swabs (oranje dop) en 2 lotnummers droge swabs (blauwe dop)

Bericht:

In het Labinf@ct *B. parapertussis* van 3 juni 2021 werd melding gemaakt van een fout-positief signaal in de *B. parapertussis*-PCR bij gebruik van e-swabs. Wij deden in dat Labinf@ct een oproep aan de laboratoria om de bevindingen van het gebruik van deze swabs te delen.

De informatie die wij verkregen, en die werd gedeeld door de medisch moleculair microbiologen in de WMDI (werkgroep moleculaire diagnostiek van infectieziekten), leidde ertoe dat *IS1001*-positieve wattentips zijn gevonden bij Copan swabs zowel in afnamesets met een oranje dop als in droge swabs (blauwe dop) :

- E-swabs met oranje dop van Copan: Er zijn in totaal door 6 laboratoria 8 verschillende lotnummers gemeld met een fout-positieve reactie: lotnummers lot# 21087100; 203038000; 202323100; 202433300; 203193300; 201896300; 202011501; 2031933.
- (Droge) swab met blauwe dop van Copan: lot# 2004732; 202472700.

Het gaat bij de fout-positieve reactie om detectie van het *IS1001*-element; de Ct-waarden liggen boven de 34; het *IS1002*-element lijkt geen reactie te geven.

Er zijn door ten minste 3 laboratoria uitslagen als positief gerapporteerd aan de inzenders. Alleen als er sprake is van een bijpassend klinisch beeld is een positieve PCR-uitslag voor *B. parapertussis* meldingsplichtig. Ook als er sprake is van meerdere gevallen in een instelling kan er een meldingsplicht (artikel 26 Wet publieke gezondheid) bestaan. Voor de complete meldingscriteria, zie [LCI-richtlijn Kinkhoest](#).

Een aantal gemelde casussen is op basis van fout-positiviteit herroepen. Bij een deel van de andere casussen is niet meer te achterhalen of de melding terecht was of niet. Deze zullen dan niet worden herroepen.

De leverancier (MLS) en producent (Copan) en de IGJ zijn geïnformeerd. Hoeveel patiënten ten onrechte zijn behandeld, is ter beoordeling aan de IGJ. De omvang van het probleem is niet vast te stellen aan de hand van de gemelde casussen, omdat een deel niet hoeft te zijn gemeld.

De leverancier MLS heeft inmiddels, namens Copan, een brief gestuurd naar de gebruikers waarin zij melden ook een positief resultaat te hebben gevonden in reeds onderzochte swabs

met oranje dop, waarbij wel/geen detectie afhankelijk is van het gebruikte platform. Daarin wordt vermeld:

*Copan cannot exclude the presence of traces of nucleic acid materials into the eSwab that may be detected as *Bordetella parapertussis* under certain testing conditions.*

As a good clinical practice, diagnostic companies and end users are required to verify the compatibility of the transport system with the specific molecular assay before use.

[...]

Copan became aware of this combination during the ongoing investigation.

Samenvattend zijn de wattenstokken gedurende het productieproces gesteriliseerd middels ioniserende straling. Zie [ESwab-Package-Insert.pdf \(copanusa.com\)](#). Kweken zijn negatief, maar DNA-resten zijn platform-afhankelijk aantoonbaar gebleven.

Contactgegevens

- **Overleg met uw regionale GGD** over casuïstiek over mogelijk verdachte gevallen in uw regio. Het nummer is te vinden op [www.ggd.nl](#).
- **RIVM-LCI:** tel. 030-2747000 (ook buiten kantooruren bereikbaar)
- **RIVM dd. arts-microbioloog:** IDS-ddmicrobioloog@rivm.nl tel. 030-2743487 (ook buiten kantooruren bereikbaar)

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Bericht verstuurd aan: leden Labinf@ct



Labinf@ct: *B. parapertussis* (2)

July 20th 2021

This letter:

- Multiple batches of swabs involved in false-positive results for *B. parapertussis*-PCR: 8 batches e-swabs (orange cap) and 2 batches dry swabs (blue cap).

Notification:

In the Labinf@ct *B. parapertussis* of June 3rd 2021, we reported that the use of e-swabs can lead to false-positive *B. parapertussis*-PCRs. We requested laboratories to share their experiences and findings with us.

The information which we received, and which was shared by the Medical Molecular Microbiologists in the WMDI (working group Molecular Diagnostics in Infectious diseases), led to the identification of *IS1001*-positive swab tips in Copan swabs. These Copan swabs were found both with orange caps (sampling set) and with blue caps (dry swabs).

- E-swabs with orange cap: Six laboratories reported 8 different batch numbers with false-positive PCRs: lot# 21087100; 203038000; 202323100; 202433300; 203193300; 201896300; 202011501; 2031933.
- (dry) swabs with blue caps: lot# 2004732; 202472700.

All false-positive PCRs concern the detection of the *IS1001*-element; Ct values typically are Ct34 or higher. The *IS1002*-element has not been detected up to now.

At least three laboratories have reported positive results to physicians. Notification of *B. parapertussis*-PCRs are only obligatory in cases with pertussis-like symptoms. Additionally,

notifications may be obligatory if multiple cases are found within an institution (article 26, law on public health). For the full list of criteria, see the LCI guideline Pertussis.

Several of the notified cases have been retracted due to false-positive PCRs. In other cases, the possibility of a false-positive PCR cannot be investigated. Consequently, these notifications will not be retracted.

The distributor (MLS), producer (Copan) and the national health authority have been informed. The national health authority will investigate whether patients have incorrectly received treatment. The extent of the problem cannot be determined based on the notifications, as notifications are not mandatory for all positive PCRs.

All e-swab users have received a letter from the distributor MLS and Copan stipulating that Copan has confirmed the possibility of false-positive PCRs on swabs with an orange cap. Moreover, the false-positivity depends on the sensitivity of the used kit and platform. The letter further mentions:

*Copan cannot exclude the presence of traces of nucleic acid materials into the eSwab that may be detected as *Bordetella parapertussis* under certain testing conditions.*

As a good clinical practice, diagnostic companies and end users are required to verify the compatibility of the transport system with the specific molecular assay before use.

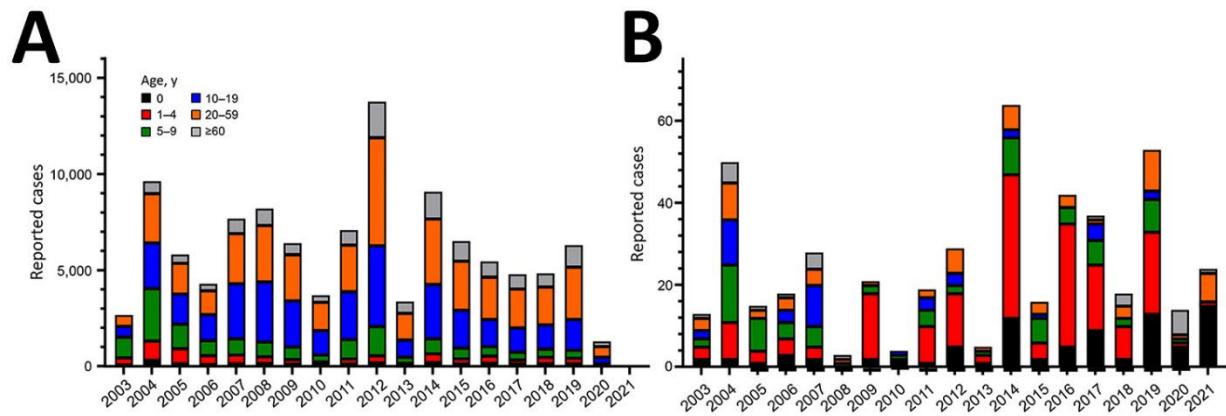
[...]

Copan became aware of this combination during the ongoing investigation.

As part of the production process, e-swabs are sterilised with ionizing radiation. See: [ESwab-Package-Insert.pdf \(copanusa.com\)](#). In line with this, all bacterial cultures are negative, however DNA remains detectable depending on the kit and platform used.

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Sent to: members of Labinf@ct



Appendix Figure. Comparison of notified cases of *Bordetella pertussis* and *B. parapertussis* registered by the National Institute for Public Health and the Environment (RIVM), the Netherlands, 2003–June 2021. A) *Bordetella pertussis*; B) *B. parapertussis*. Annual reported cases are stratified by age groups.